EXTRACTION OF ANTIOXIDANT ACTIVITY, PHENOLIC CONTENT AND MINERALS IN BANANA PEEL

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ABSTRACT

The objective of this research project is to establish the optimal conditions for obtaining the banana peel extract with high antioxidant activity, phenolic content and minerals. The main by-product of the banana processing industry is the peel, which represents approximately 30% of the fruit. The banana peel extraction process was optimized by varying different parameters such as drying temperature for banana peels, types of solvent used, various type of solvents ratio, temperatures of extraction and time of extraction. The drying process was done at temperatures of 50^{o}C - 70^{o}C for 24 hours. The ground banana peel samples were extracted with different solvents (methanol, acetone and distilled water) with different solvents ratio (50%, 70% and 90%). The extraction was performed in a water bath at 40^{o}C-60^{o}C for 1- 120 min. UV-visible spectrophotometer was used to determine the antioxidant activity and phenolic content. High Performance Liquid Chromatography (HPLC) used to verify the existence of vitamin C. The minerals composition was determined by using atomic absorption spectrophotometer. The result showed that, optimal condition for the extraction of antioxidant activity, phenolic content and minerals in banana peel were drying at 60^{o}C, extracted by using 70% acetone solvent in the water bath for 2 hours at 55^{o}C. Concentration of antioxidant activity and phenolic content at this optimal extraction condition was 1061.33±0.03μmol/g and 1474.17±0.02 mg/L respectively.

ABSTRAK

Tujuan projek ini adalah untuk mendapatkan keadaan optimum untuk mendapatkan ekstrak kulit pisang dengan aktiviti antioksidan, kandungan fenolik dan mineral yang tinggi. Bahan buangan utama dari industri pemprosesan pisang adalah kulit pisang, yang mewakili sekitar 30% dari buah. Proses ekstraksi kulit pisang adalah dioptimumkan oleh pelbagai parameter yang berbeza seperti suhu pengeringan kulit pisang, jenis pelarut yang digunakan, nisbah pelarut, suhu ekstraksi dan masa ekstraksi. Proses pengeringan dilakukan pada suhu 50^{o}C - 70^{o}C selama 24 jam. Sampel pisang tersebut diekstrak dengan mengunakan pelarut yang berbeza (metanol, aseton dan air suling) dengan perbandingan pelarut yang berbeza (50%, 70% dan 90%). Ekstraksi dilakukan dalam penangas air pada suhu 40oC-60oC selama 1 - 120 minit. Spektrofotometer UV digunakan untuk menentukan aktiviti antioksidan dan kadar fenolik. Kromatografi (HPLC) digunakan untuk membuktikan kewujudan vitamin C. Komposisi mineral ditentukan dengan menggunakan spektrofotometer serapan atom. Keputusan kajian menunjukkan bahawa, keadaan optimum untuk ekstraksi aktiviti antioksidan, kandungan fenolik dan mineral dalam kulit pisang adalah pengeringan 60oC, diekstraksi dengan menggunakan pelarut aseton 70% dalam air selama 2 jam pada 55^{o}C. Konsentrasi aktiviti antioksidan dan kadar fenolik pada keadaan ekstraksi yang optimum adalah 1.061,33 ± 0.03μmol / g dan 1474.17 ± 0.02 mg / L masing-masing.

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LIST OF SYMBOLS

^{o}C	Degrees Celsius
%	Percentage
µM	MicroMolar
µL	Micro liter
µmol/g	micromol per gram
HCl	Hydrocloric Acid
m	Meter
m^3	Cubic meter
mg	miligram
mg/l	miligram per Liter
min	minute
mL	mililiter
mL/min	milliliter per minutes
mM	miliMolar
mol/g	mol per gram
nm	nanometer
O_2	Oxygen
rpm	rotation per minutes
ppm	part per million
w/v	Weight per Volume
g	gram
h	hour

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CHAPTER 1

INTRODUCTION

1.1 Research Background

According to several authors, banana peel recorded stronger antioxidant activity, pooled more quantity of phenolic compounds (Someya *et al.*, 2002), greater range of phenolics composition and higher in minerals content than banana pulp. Banana pulp had been reported as having various antioxidants such as vitamins (A, B and E), β – carotene (Kanazawa & Sakakibara, 2000) and phenolic compounds like catechin, epicatechin, lignin, tannin (Someya *et al.*, 2002), gallocatechin and anthocyanins like peonidin and malvidine.

There several type of antioxidant such as vitamin C, E, A, beta-carotene, lycopene and also other substances. Antioxidants are abundant in fruits and vegetables, as well as in nuts, grains, and some meats, and fish. Combination of vitamin C with other antioxidants, including vitamin E, b-carotene, and selenium, provides a synergistic antihypertensive effect. Experimental evidences prove that antioxidants can protect human body from free radicals and reactive oxygen species (ROS) effects.

Antioxidants is substance that can prevent or slow the oxidative damage process toward our body. Besides, antioxidant also protect cells from the damage that may caused by free radicals. Free radicals are unstable molecules or highly reactive chemical that often contains oxygen and is produced during oxidation. The defensive effect of natural antioxidant in fruit and vegetable are related to its major group, which are vitamin, phenolic and carotenoid.

Ascorbic acid and phenolic and are known as the hydrophilic antioxidant while carotenoid was known as lipophilic antioxidant (Halliwell., 1996). Exposure to various environmental factors will lead to free radical formation such as tobacco smoke and radiation. Free radicals can damage important cellular molecules such as DNA or lipids or other parts of the cell and may lead to cancer.

Banana peel also demonstrated the presence of various phenolic compounds such as gallocatechin and anthocyanins like peonidin and malvidin. Phenolic compounds are secondary metabolites, which have been associated with flavour and colour characteristics of fruits and vegetables and are gaining considerable attention because of their potent antioxidant and health promoting properties (Kaur & Kapoor, 2001). Extraction of phenolic compounds in plant materials is influenced by many factors including the extraction method employed, types of solvent polarity used, storage time, presence of interfering substances, sample particle size and conditions as well as their chemical nature.

Bananas were enriched with minerals like potassium, phosphorus, magnesium and calcium. Banana peel could be a good source of carbohydrates and fiber. The high fiber content also indicates that the peels could help treat constipation and improve general health and well being (Anhwange, B. A. *et al.,* 2009*).*

Natural antioxidant are compound from plant or animals sources that retard oxidative rancidity of oil, fats and fat soluble components, thus protecting them while delaying the development of unpleasant flavors and odors resulting from oxidation process.

Antioxidants are present naturally in most raw food sources. Processing of the fruit can remove or trigger the degradation of this antioxidant.

Nowadays, the most widely used antioxidant were synthetic antioxidant such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butylhydroxyanisole (TBHQ), and propyl gallate (PG). Figure 1.1 shows the molecular structure of the available synthetic antioxidant.

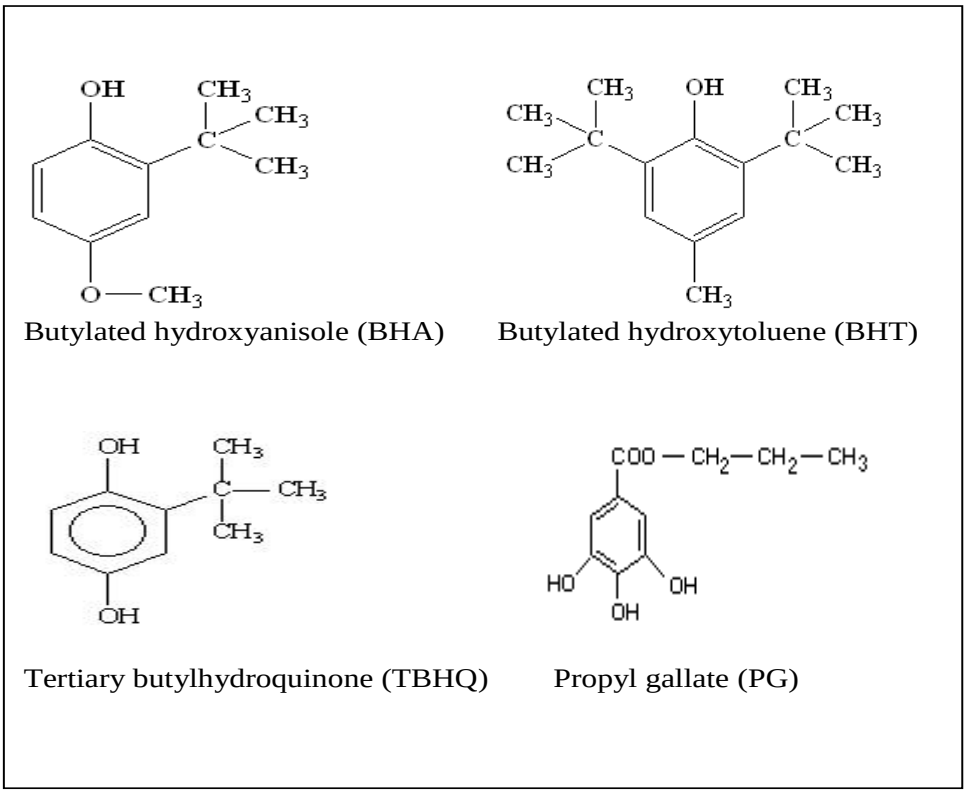

Figure 1.1: Synthetic antioxidants

1.2 Problem Statement

Doubt on the safety of the synthetic arose first in the 1960s and led to an increased interest and broad research on natural antioxidant. Natural antioxidant particularly found

in fruit and vegetables have gain interest among consumer and the scientific community because epidemiological studies have indicate that frequent consumption of natural antioxidant is associate with the lower risk of cardiovascular and cancer (Renoud et al.1998).

Natural antioxidants are perceived safe, less toxic and beneficial for human health. However, natural antioxidant is very expensive and has been not widely commercialized. Recently, the demand for natural antioxidants has increased, due to consumer concerns about the safety of synthetic antioxidants (S. Okonogi et al., 2007).

There had been an explosive interest in studying antioxidants of some fruits due to their health promoting properties. A large number of studies have been demonstrate either on the effect of extraction time and extraction temperature or drying temperature toward antioxidant activity, phenolic content and minerals in banana peels.

Antioxidant activity could influence by geographical origin, cultivar and harvest storage time. *(*C. Guo *et al., 2003).* These statements show that different origin of banana peel will have different value of antioxidant. This study is focusing on the banana peel that originally harvest in Malaysia because banana peel from Malaysia origin is rarely being studied before.

1.3 Objective

The objective of this research project is to establish the optimal condition for obtaining the banana peel extract with high antioxidant activity, phenolic content and minerals by using solvent extraction.

1.4 Research Scope

Following tasks will be undertaken as a part of the proposed research:-

- Different drying temperatures of 50^{o}C, 55^{o}C, 60^{o}C, 65^{o}C and 70^{o}C used to dry the sample. 70% acetone was use as the extracting solvent.
- The most optimum drying temperature sample was extract by using different type of solvent (methanol, acetone and water) with different solvent ratio (90%, 70%, 50%). The extraction was done at 50^{o}C for 1 hour in the water bath.
- Time of extraction vary from 1min, 30min, 60min, 90min and 120min was used to determined the effect of extraction time on antioxidant activity and phenolic content. Optimum drying temperature of the banana peel sample and optimum solvent ratio was used in the extraction process.
- Effect of different extraction temperatures of 40^{o}C, 50^{o}C, 55^{o}C and 60^{o}C were analyst where optimal conditions that obtained in the drying temperature, type of solvent, solvent ratio and time of extraction were used in the extraction process.
- Antioxidant activity and phenolic content was analyst by using Ferric Reducing Antioxidant Power (FRAP) assay and Folin-Ciocalteu's calorimetric (FC) respectively. The detection of the products was performed by using UV-visible Spectrophotometer.
- Verification of the Ascorbic acid existence in the banana peel extract at optimal conditions was performs using High Performance Liquid Chromatography (HPLC) unit.
- Sodium(Na), Magnesium(Mg) and Calcium(Ca) compositions in banana peel extracted at optimal condition were investigate by using Atomic Absorption Spectrophotometer (AAS) unit.

1.5 Rationale and Significance

Banana peels are waste from banana fruit and they contain high amount of antioxidant, phenolic content and mineral. This peel is biodegradable and it will produce environmental problem due to it nitrogen and phosphorus quantity. Therefore, extracting the banana peel will be the best solution in order to protect human being, gaining some profit and creating waste to wealth. Banana peel also can be commercialize because it qualitative and quantitatively contain more antioxidant than it pulp. It also will have does not compete with banana pulp in producing end product especially in the food industry. Besides, banana fruit is easy obtains because it is not a seasonal fruit and it grow well in Malaysia. These ensure that, banana can continuously act as the natural antioxidant source.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

2.1.1 Banana (Banana Peel)

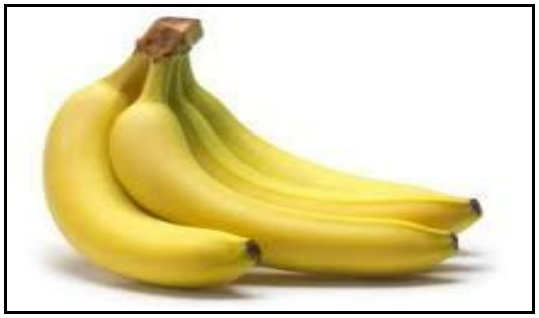

Figure 2.1: Banana Fruit (*Musa sapientum)*

Banana is genus of *Musa* and family of *Musaceae.* It was originally from Malaysia and are now cultivated all over the tropical and sub-tropical continents. Banana plants are the world's biggest herbs, grown abundantly in many developing countries (G. Aurore et al., 2009). Musa sapientum trees are best cultivated in a highly organic soil with pH 5.5-7.0. This plant need a lot of water is to grow and yield bananas.

After 9-12 month of planting the banana plant, flower start to develop. The underground stem initiates the development of the flower. The stem is also known as pseudostem. After producing single bunch of banana, this pseudostem will die and replace with new pseudostem. Banana plant can grow up to the height of 2-8m. Fruit maturation take an about 60 - 90 days after flowers first appear. The banana fruit grows in hanging cluster, with twenty fruits to a tier and 3 - 20 tiers to a bunch. The fruit is protected by its peel which is discarded as waste after the inner fleshy portion is eaten (Anhwange, B. A. et al., 2009).

The primary reason for the cultivation of banana plant is its fruits. Banana fruits are widely available and they had been used as food without apparent toxic affect. In some countries, banana fruit and its peel are considered to be the golden fruit of nature because they do helps to promote natural beauty by providing the body with essential nutrients and also healthy digestion. It have been reported that banana fruit do help preventing anaemia, cure the heart burns stress, strokes, ulcers and many other minor illness. Banana should be considered to be a good source of natural antioxidant for foods and functional food source against cancer and heart disease (Someya, S., Y. Yoshiki and K. Okubo, 2002).

Peels are often the waste part of various fruits. These wastes have not generally received much attention with a view to being used or recycled rather than discharged. This might be due to their unknown benefit of commercial application. The main by-product of the banana processing industry is the peel, which represents approximately 30% of the fruit. This by-product constitutes an environmental problem because it contains large quantities of nitrogen and phosphorus and its high water content makes it susceptible to modification by microorganisms (R. González-Montelongo et al., 2010). Interestingly, the peel and seed fractions of some fruits have higher antioxidant activity than the pulp fractions

The banana peel could be a potential being the source of antioxidant and antimicrobial activities. Banana peel is rich in phytochemical compounds, mainly

antioxidants (R. González-Montelongo et al., 2010). Banana peels are commonly used as a home remedy for several skin problems including allergies, bruises and skin irritation. It can reduced several skin problems conditions including treating acne, treating poison ivy rashes irritation from mosquito bites, reducing bruises, getting rid of warts and also managing wrinkles. Banana peel will not be instantly effective to manage the wrinkles, but it helps the skin to look more radiant, fresher and healthier.

Potential applications for banana peel depend on its chemical composition. Banana peel is rich in dietary fibre (50% on a dry matter (DW) basis), proteins (7% DW), essential amino acids, polyunsaturated fatty acids and potassium. Banana peel is rich in phytochemical compounds, mainly antioxidants. The total amount of phenolic compounds in banana peel ranges from 0.90 to 3.0 g/100 g DW .

Someya et al. (2002) identified gallocatechin at a concentration of 160 mg/100 g DW in the banana peel. Ripe banana peel also contains other compounds, such as the anthocyanins delphinidin and cyaniding, and catecholamines. Furthermore, carotenoids, such as b-carotene, a-carotene and different xanthophylls, have been identified in banana peel in the range of 300–400 lg lutein equivalents/100 g, as well as sterols and triterpenes. To date, only Someya et al. (2002) have evaluated the antioxidant activity in banana peel, measured as the effect on lipid autoxidation in relation to its gallocatechin content. (R. González-Montelongo et al., 2010)

Banana peels have been effectively used for the synthesis of silver nanoparticles. Currently, silver nanoparticles is use in the production of antibacterial and antifungal agents biotechnology and bioengineering, textile engineering, water treatment, and silver-based consumer products. It can be synthesized by several chemical, physical and biological method. Nanoparticle are one of the effective medium in against the fungal and bacteria culture such as C. albicans and E. coli, Staphylococcus aureus, Bacillus anthracis and Proteus mirabilis. These in turn, could be applied in the fields of microelectronics, biodiagnostics, sensing, and imaging as well as in designing newer drugs (A. Bankar et al.,*2010).*

The commonly used of banana peel in the silver nanoparticles production is due to the its composition. Banana peels are inherently rich in polymers such as lignin, hemicellulose and pectins that contribute to the synthesis of silver nanoparticles. (A. Bankar et al.,2010). Therefore, banana peel is a potential new generation for the production antimicrobials products due to displayed by of antimicrobial activities silver nanoparticles that produced from the banana peels.

2.2 Antioxidant Activity And Phenolic Content

Bananas peel are a good source of natural antioxidants, which include vitamins and beta carotene**.** It is contains a number of antioxidants and minerals that can help the skin restore itself naturally. Natural antioxidant are primarily phenolic compound that may occur in all part of a plant. They are multifunction and can react as free radical terminators, metals chelators and single oxygen quenchers. The common plant phenolic antioxidant are topopherols, flavonoids and other related compound such as phenolics acid.

Antioxidants react as free radical scavengers by protecting the cell from damage by free radicals .Antioxidants may also enhance immune defense. In humans, the most common form of free radicals is oxygen. Oxygen molecule (O_2) steals electrons from other molecules when it is electrically charged. This may caused damage to the DNA and other molecules. Over time, such damage may become irreversible and lead to disease including cancer.

Free radicals can attack any various substrates of interest in the body,. Therefore, it will contribute to chronic disease development such as oxidatively modified LDL. Oxidatively modified LDL has been hypothesized to be a causative agent in the

development of cardiovascular disease . Oxidatively modified DNA may also play an important role in human carcinogenesis. Many nutritional factor are widely considered to be critical for human health. Among them, free radicals have been of concern as one of the factors contributing to chronic degenerative disease (O. Patthamakanokporn *et al.*, 2008).

Antioxidants protect fats and lipids in foodstuffs. Most of these act through reaction with free radicals. Formation of these free radical from lipids is the first step in their oxidative deterioration. Other antioxidants in food such as ascorbic acid which lower the potential of the foodstuffs to which they are added. Oxidation reaction has deleterious effect on the antioxidant activity where this oxidation level is influenced by temperature, light, air, physicochemical as well as the presence of catalyst (Frankel&Meyer, 2000)

Phenolic compounds are in the category of natural antioxidants and are the most abundant antioxidants in human diet. Phenolic make an important contribution to the flavor to the fruits It is well known that fruits contain various antioxidants, such as vitamin C, vitamin E, and b-carotene. Phenolic compounds are naturally and commonly found in both edible and inedible plants. The phenolic content and composition in plants depend on genetic and environmental factors, as well as post harvest processing and storage conditions. The recovery of polyphenols from plant materials is influenced by the solubility of the phenolic compounds in the solvent used for the extraction process. This prove that solvent polarity will play a key role in increasing phenolic solubility.

Folin-Ciocalteu’s calorimetric (FC) method is the method to analyze the phenolic content in the banana peel extracts. For qualitative identification of phenolic compounds, ultraviolet– visible spectrophotometer were considered as a tool for the identification of the phenolic contents.

2.3 Minerals Profile

Minerals play a vital role in proper development and good health of the human body and fruits are considered to be chief source of minerals needed in the human diet. Inadequate intake of mineral has been observed to be a major nutritional problem in our environment.

Banana has been reported to prevent anaemia by stimulating the production of haemoglobin in the blood. Its role to regulate blood pressure has been assosiated eith the high content of potassium. Its peels in conjunction with other substances create a liniment for reducing the acuteness of the arthritis aches and pains**.**

Both banana peel and pulp have impressive potassium content. It is highly recommended by doctors for patients whose potassium is low. B. A. Anhwange (2008) states as in table 1.1 that concentration potassium in the banana was found to be highest (78.10 mg/g) among other minerals compound. The concentration of calcium, sodium, iron and manganese are 19.20, 24.30, 0.61 and 76.20, respectively. The minerals profile are determined by using flame atomization.

Table 2.1: Mineral composition of *Musa sapientum peel*

Element	Concentration (mg g^{-1})
Potassium	78.10±6.58
Calcium	19.20±0.00
Sodium	24.30±0.12
Iron	0.61±0.22
Manganese	76.20±0.00
Bromine	0.04±0.00
Rubidium	0.21±0.05
Strontium	0.03±0.01
Zirconium	0.02±0.00
Niobium	0.02±0.00

2.4 Vitamin C

Another abundant of antioxidant in nature is ascorbic acid. Ascorbic acid or Vitamin C is organic acid with antioxidant properties and also water-soluble compound that fulfills several roles in living systems. It is generally recognized as safe substances by FDA. As a potent antioxidant, ascorbic acid has the capacity to eliminate several different reactive oxygen species, keeps the membrane-bound antioxidant a-tocopherol in the reduced state, acts as a cofactor maintaining the activity of a number of enzymes (by keeping metal ions in the reduced state), appears to be the substrate for oxalate and tartrate biosynthesis and has a role in stress resistance (Y. Hernández et al., 2006).

Ascorbic acid serves as a reducing agent and may exert a chelating action. Several analytical methods have been reported for the determination of vitamin C using titrimetry, spectrometry and amperometry .Most of these methods may give overestimes due to the presence of oxidizable species other than AA and/or not to measure DHA. For example, the AOAC's official method , based on the titration of AA with 2,6-dichloroindophenol in acidic solution, is not applicable in all the matrices (Y. Hernández et al., 2006). The commonly equipment used to the determined the ascorbic acid composition is by using high performance liquid chromatography (HPLC).

The major benefit of ascorbic acid with regard to cancer is it reacts as anticancer agent. It may be precaution from developing cancer, rather than in therapy. Vitamin C work from inside the cells and protect DNA, the hereditary material in cells from the damage caused by free radicals. It can also reduce the development of nitrosamines from nitrates. Nitrates chemicals that are commonly used for foods processing. Once formed, nitrosamine can become carcinogenic which can cause cancer.

Lea (1992) reported that fresh apple contains up to 100ppm of vitamin C but during the processing into juice it is rapidly lost. The lost of ascorbic acid was also found to be

highest in the medicinal plant dried at 50^{o}C for 9 hours (75.60%) compared to freeze drying(21.13%)(Mahanom et al. 1999). This statement show that, the amount of ascorbic acid exist in the is depend on the it processing method.

2.5 Analysis Method

2.5.1 Solvent Extraction

The most common techniques that were employed to obtain high yield of antioxidant activity is direct by using solvent. The solvent used for the extraction is a major importance for the recovery of the antioxidant component, the coextraction of undesirable substances and the process yield. Selective extraction methods should be practiced since active compounds in plants that exhibit biological activities are usually present in low concentrations.

The type of the solvent used to extract antioxidants from banana peel can affect single electron transfer and hydrogen atom transfer, which are key aspects in the measurements of antioxidant capacity. The polarity of the solvent and that of the different antioxidant compounds affects the efficiency of the extraction and the activity of the obtained extracts. Water, methanol, ethanol, acetone, aqueous solutions of the aforementioned solvents and ethyl acetate are commonly used as extraction solvents (R. González-Montelongo et al., 2010). However acetone is the most commonly used as extraction solvent due to it extensive validation by FDA.

Several studies focused on the efficiency of different organic solvent used in extraction process. Organic solvent with high polarity are more effective in quantitive recovery than nonpolar solvent. For example, the extraction of various aromatic herbs by